Development of Standards for Cation Exchange Chromatography Column Qualification

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Introduction

Standards for qualification of chromatographic columns were identified as a high priority during USP-sponsored roundtables with stakeholders to identify challenges in biologics development that could be alleviated with standards. Cation Exchange Chromatography (CEX) analysis is commonly used to characterize the charge heterogeneity of therapeutic proteins by determining acidic and basic charge variants. Salt gradient and pH gradient are two widely used methods of CEX analysis. The aims of this proof-of-concept study were to establish both pH gradient and salt gradient CEX methods and utilize them to evaluate three USP monoclonal antibody standards in development (USP mAb001, USP mAb002 & USP mAb003) on columns from three different vendors. Resulting profiles and peak resolution were evaluated to select a candidate for further development as a standard for CEX column qualification.

Materials and Methods

Materials:

Table 1: USP mAbs for CEX column qualification

Methods:

- pH gradient method
- Salt gradient method
- Selection of starting point for method development
- Optimise/evaluate the selected method using USP mAbs
- Further evaluates charge variant profile of USP mAbs on columns from different vendors

Figure 1. Workflow for evaluation of USP mAbs for CEX column qualification standards

Results

Part I: by pH Gradient Method

Figure 2. Profiles of USP mAb001 (A), USP mAb002 (B) and USP mAb003 (C) on different columns by pH gradient method

Table 4: Reproducibility and relative percentage of charge variants by pH gradient method

- Table 5: Reproducibility and relative percentage of charge variants by salt gradient methods

- Table 6: Profiles of USP mAb001 (A), USP mAb002 (B) and USP mAb003 (C) on different columns by salt gradient method

Conclusions

- Generic pH gradient and salt gradient CEX methods were established and used to evaluate the charge variant profiles for three USP mAbs on columns from 3 different vendors, including two SCX columns and one WCX column
- Better resolution of charge variants on SCX columns was observed, suggesting further optimization may be needed for CEX columns
- Peak fronting was observed for USP mAb003 on all three columns
- The percentages of Main, Acidic and Basic peaks were similar across columns for each mAb
- USP mAb001 was selected as the top candidate for further development. This mAb was prioritized because:
  - Four acidic and multiple basic charge variants could be resolved by both pH gradient and salt gradient CEX chromatography
  - It yielded a consistent charge variant profile across the 3 columns tested
- Next steps include identification of individual peaks and testing on additional columns

Note: USP mAbs standards will be released in Spring/Summer 2020

References